EVALUATION OF MEAT QUALITY IN TELEORMAN BLACK HEAD BY ECOGRAPHIC METHOD AND IDENTIFICATION OF CALPASTATIN (*CAST*) GENE POLYMORPHISM CORRELATED WITH CARCASS QUALITY

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Abstract

The objective of this study was to evaluate meat quality using ultrasound method measuring two points in Longissimus Dorsi muscle and the identification of CAST gene correlated with carcass quality at 105 lambs of Teleorman Black Head breed. There was determinate the fat depth in males (2.33, 2.51 mm) and females (2.12, 2.11mm), muscle depth (22.45, 22.68 mm) and (20.69, 20.95 mm), eye muscle area (9.28, 9.30 cm^2) and (8.91, 8.87 cm^2). Medium correlations where found in these two points between birth weight with muscle depth (0.45, 0.49) and eye muscle area (0.40, 0.54). Medium to high correlations resulted between 3.5 month weight with muscle depth (0.58, 0.65) and with eye muscle area (0.55, 0.67) and also average daily gain with fat depth and with muscle depth given medium to high correlations (0.51, 0.44, 0.46, and 0.59). Between muscle depth and eye muscle area in this two measurement points resulting high correlations (0.71, 0.76) also between muscle depth and eye muscle perimeter (0.90, 0.85) revealing lambs meat potential. For calpastatin, was extracted blood DNA and polymorphism was determined by PCR RFLP. The PCR products were digested with Hae III enzyme. Calpastatin gene frequency in these tree genotypes AA, AB and BB was 73.68 %, 6.32% and 23%. The allele A frequency was 77 % and for allele B was 23%. CAST gene can be a useful tool in marker assisted selection for meat quality.

Key words: carcass quality, Longissimus Dorsi, calpastatin gene, sheep

INTRODUCTION

Worldwide meat production is a requirement that must be accomplished, given the growing demand of human population globally, but also at the European level. Sheep meat is a staple food with a remarkable biological value and therefore Romania should take into account this great potential that is mostly guiding this direction, with great opportunities to regain sheep meat domestic market of Asia. High quality carcasses are the prerogative of countries with a long tradition in sheep breeding for meat production as France, Spain, England, Czech Republic, Italy and also Romania. The quality of sheep carcasses is emphasized by modern methods, such as ultrasound method witch highlighting *Longisimus Dorsi* muscle, very well correlated with a large amount of meat in the carcasses [6]. Also another method of investigation with great effect in marker-assisted selection is molecular markers correlated with the amount of meat in the carcasses. There are studies that have been done on calpastatin ([1], [4], [3]) gene with a role in meat production and meat quality. Calpastatin nature is endogenous and it is a specific inhibitor of the protease calpain that regulates the rate and degree of tenderization of meat after slaughter [7]. An increased rate of skeletal muscle development may be the result of a decrease in muscle protein degradation due to a reduced calpain activity and a growth of calpastatin (*CAST*) activity.

The objective of this study was to evaluate meat quality using ultrasound method measuring four parameters (fat depth, muscle

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The manuscript was received: 31.08.2015
Accepted for publication: 10.09.2015

depth, eye muscle area and eye muscle perimeter) in two points in *Longissimus Dorsi* muscle and the identification of *CAST* gene polymorphism correlated with carcass quality at 105 lambs of Teleorman Black Head breed (TBH).

MATERIAL AND METHODS

Animals. The study was conducted on 105 Teleorman Black Head (TBH) lambs aged 3.5 months, in a farm from Teleorman County (Romania). Body weight at birth (BW) and body weight at (WE) 3.5 months were measured in order to determine daily gain (DG) and average daily gain (ADG).

Ultrasound measurements. The ultrasound measurements have been performed with an Echo Blaster 64 with LV 7.5 65/64 probe (TELEMED ultrasound medical systems, Lithuania). All ultrasound images were recorded and analyzed with Echo Wave II 1.32, software. The first measuring point (P1) was located at 5 cm from the spine, in line with the 12th rib; the second measuring point (P2) was located between lumbar vertebrae 3 and 4. A large proportion of LD muscle is situated between these two measuring locations and this provides information on the parameters which are important for the evaluation of meat production in lambs: fat layer depth (F12 F34), muscle depth (M12, M34), muscle eye area (A12, A34) and muscle perimeter (P12, P34) at rib 12th and between 3 and 4 lumbar vertebrae, area of LD muscle.

PCR-RFLP method was used to determine polymorphism of *CAST* gene. Blood samples were extracted from 105lambs and the PCR products were digested with *Hae III* enzyme. PCR analyses were done using primers for specific amplification of *CAST* gene:
Fw: TGGGGCCCAATGACGCCATCGATG
Rv: GGTGGAGCAGCACTTCTGATCACC [10].

Mix PCR total volume 7.7 µl: 4µl PCR tampon, 2 µl $MgCl_2$, 0.4 µl dNTP, 0.6 µl primers, and 0.1 µl Go Tak DNA polymerase. **PCR program**: 10 minutes denaturation at 95^{O}C, 40 cycles with 30 seconds denaturation 95^{O}C, 30 seconds elongation at 56^{O}C, 30 seconds 72^{O}C extension, and final extension 10 minutes at 72^{O}C and then decreasing at 4^{O}C for being kept on conservation until is used in RFLP reaction. *CAST* gene amplicon of 622 bp was visualized on 2 % agarose gel, DNA ladder was 100bp. **RFLP program for *CAST*** identification used was: 180 minutes at 37^{O}C using a RFLP mixture with a total volume of 5 µl: 2 µl Buffer C, 0.5 µl BSA, 0.5 µl Hae III, and 2 µl ultra-pure water, total volume of the reaction: 20 µl with 15 µl of CAST amplicon and 5 µl RFLP mixture. RFLP products after digestion was visualized on 4% agarose gel.

RESULTS AND DISCUSSION

Evolution of lambs body weight in the present study follows (83 females and 22 males) the standards of TBH sheep breed ([5], [8]) with 6.01 kg for males and with 8.83% less on females than on males ($P< 0.01$). Average weight at 3.5 months age was 33.51 kg in males and 14.86% higher than that recorded by the females with highly significant difference ($P<0.001$). Similar studies were done and Walsh mountain lambs recorded a 38.85% smaller weight at 3.5 month age than TBH lambs [2] and Chura Galega Bragancana lambs was 7.43% heavier than TBH lambs [12]. Average daily gain has the same trend and it recorded on males and females 0.26 kg and 0.20 kg respectively ($P<0.001$) (table 1).

Table 1 Body weight evolution, average daily gain and age at Teleorman Black Head (83 female, 22 males)

Item	sex	X ±s	CV (%)
Birth weight (kg)	M	6.01 ± 0.11	8.83
	F	5.47 ± 0.09	15.40
Weight experiment (kg)	M	33.51 ± 0.79	11.04
	F	28.53 ± 0.71	22.73
Daily gain (kg)	M	27.51 ± 0.73	12.48
	F	23.07 ± 0.65	25.75
Average daily gain (kg)	M	0.26 ± 0.01	19.11
	F	0.20 ± 0.01	31.75
Age (days)	M	109.23 ± 4.70	20.19
	F	120.31 ± 3.40	25.77

BW-birth weight P<0.01 DS, WE-experiment weight P<0.001, FS, DG-daily gain P<0.01 DS, ADG-average daily gain P<0.001 FS, Age P>0.05 NS.

Regarding the phenotypic correlations between ultrasound measurements with birth weight, 3.5 months weight, total daily gain and average daily gain, from a total of 78 traits couples 19.23% are small correlation (0.00-0.30), 41.02% are medium to high correlations (0.31-0.60) and high correlations recorded 39.75 % (0.61-1.00).

Medium correlations where found in these two points between birth weight with muscle depth (0.45, 0.49) and eye muscle area (0.40, 0.54). Medium to high correlations resulted between 3.5 month weight with muscle depth (0.58, 0.65) and with eye muscle area (0.55, 0.67) and also average daily gain with fat depth and with muscle depth given medium to high correlations (0.51, 0.44, 0.46, and 0.59). Between muscle depth and eye muscle area in this two measurement points resulting high correlations (0.71, 0.76) also between muscle depth and eye muscle perimeter (0.90, 0.85) revealing Teleorman Black Head lambs meat potential (table 2).

Regarding ultrasound parameters in these two measurements points at *Longisimus Dorsi* muscle we can see that TBH lambs are situated in the limits recommended for sheep meat production, having a fat depth in P1 of 2.33 mm in males and in females 2.12 mm smaller than other sheep breed like Chura da Terra Quente and Awassi ([11], [10]). Muscle depth in these two measurements points P1 and P2 was 22.45 and 22.68 mm in males, with 7.83% and 7.62% higher than females respectively. Similar results with muscle depth were obtained on Chura da Terra Quente (26.4 and 26.5 mm) [11]. Males eye muscle area in the present study was 9.28 cm^2 being 3.98% higher than females. In the present study eye muscle area at TBH lambs was 42 % smaller than Chura da Terra Quente [11] and higher than Awassi lambs with 14.55 % for females [10].

CAST amplicon was obtained after PCR reaction and was visualized on 2% agarose gel, it was a *CAST* DNA fragment of 622 bp (figure 1). By RFLP with *Hae III* there were obtained tree genotype variants: 70 animals-*AA*, 6 animals-*AB*, and 19 animals–*BB* from 95 animals. Genotype AA has 4 restriction fragments 447 bp, 432 bp, very closely one to each other, visualized in one single band, and another two fragments of 188 bp and 173 bp. Genotype *AB* has 5 bands: 447 bp, 432 bp, 394 bp, 188 bp and 173 bp and on 4% agarose gel it was observed only tree fragments (figure 2). Genotype *BB* was obtained after RFLP reaction and a fragment of 394 bp was observed (figure 3). *CAST* gene polymorphism showed three variants with a genotype frequency of73.68% for *AA*, 6.32% for *AB* and 20% for *BB*. Allele A frequency recorded 77%, while allele B has only 23% (table 4, figure 4).Expected and observed values of *CAST* genotypes (Table 5) show a Hardy-Weinberg disequilibrium. This disequilibrium might be caused by an empiric selection used for TBH breed detriment for heterozygous genotype. Calpastatin genotypes are in Hardy-Weinberg disequilibrium because χ^2 test was calculated and the obtained value was greater than the tabular one.

Table 2 Phenotypic correlations between body weight and echo graphic parameters at Black Head Teleorman lambs 3.5 month age

Item	BW	WE	DG	ADG	F12	F34	M12	M34	A12	A34	P12	P34
BW	1.00											
WE	0.70	1.00										
DG	0.63	0.99	1.00									
ADG	0.51	0.65	0.64	1.00								
F12	0.34	0.42	0.41	0.51	1.00							
F34	0.29	0.30	0.29	0.44	0.48	1.00						
M12	0.45	0.58	0.57	0.46	0.32	0.25	1.00					
M34	0.49	0.65	0.64	0.59	0.51	0.33	0.51	1.00				
A12	0.40	0.55	0.55	0.21	0.32	0.21	0.71	0.46	1.00			
A34	0.54	0.67	0.66	0.37	0.41	0.24	0.53	0.76	0.74	1.00		
P12	0.29	0.43	0.43	0.00	0.19	0.11	0.46	0.30	0.90	0.68	1.00	
P34	0.35	0.51	0.51	0.13	0.22	0.13	0.37	0.44	0.70	0.85	0.75	1.00

BW-birth weight, WE-experiment weight, DG-daily gain, ADG-average daily gain, F12, F34-fat depth, M12, M34-muscle depth, A12, A34-eye muscle area, P12, P34-eye muscle perimeter

Table 3 Eco graphic parameters obtained at Teleorman Black Head lambs (83 female, 22 males)

Item	sex	UM	X ± s	CV (%)
Fat depth on 12 rib	M	mm	2.33 ± 0.12	24.74
	F	mm	2.12 ± 0.06	31.26
Fat depth between 3 – 4 lumbar vertebra	M	mm	2.51 ± 0.11	19.56
	F	mm	2.11 ± 0.06	26.31
LD Muscle depth on 12 rib	M	mm	22.45 ± 0.57	11.99
	F	mm	20.69 ± 0.31	13.46
LD Muscle depth between 3 – 4 lumbar vertebra	M	mm	22.68 ± 0.49	10.06
	F	mm	20.95 ± 0.39	17.08
Eye muscle area on 12 rib	M	cm^2	9.28 ± 0.21	10.49
	F	cm^2	8.91 ± 0.18	18.01
Eye muscle area between 3 – 4 lumbar vertebra	M	cm^2	9.30 ± 0.27	13.62
	F	cm^2	8.87 ± 0.19	19.36
Eye muscle perimeter on 12 rib	M	mm	125.62 ± 1.63	6.09
	F	mm	126.65 ± 1.33	9.55
Eye muscle perimeter between 3 – 4 lumbar vertebra	M	mm	127.02 ± 2.11	7.81
	F	mm	126.07 ± 1.24	8.93

F12-fat depth P>0.05 NS; F34-fat depth P<0.01 DS; M12muscle depth P<0.01 DS; M34-muscle depth P<0.05 S; A12, A34-eye muscle area P>0.05 NS;P12, P34-eye muscle perimeter P>0.05 NS

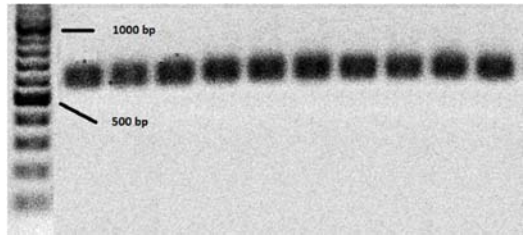

Figure 1 PCR *CAST* amplicon of 622 bp, 2% agarose gel, DNA ladder 100 bp

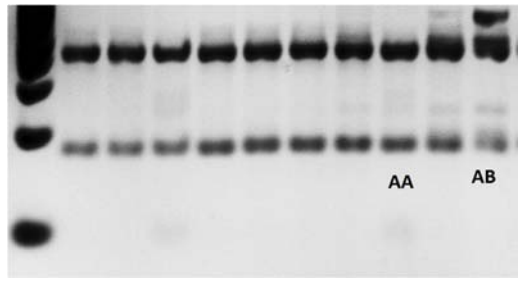

Figure 2 RFLP *CAST* 4 % agarose gel, DNA ladder 100 bp

Figure 3 RFLP *CAST*, 4% agarose gel DNA ladder 100bp

Table 4 Allelic and genotypes frequency at *CAST* gene for studied breed

Animals	Genotype %			Allele frequency	
	AA	AB	BB	A	B
95	73.68	6.32	20	0.53	0.47

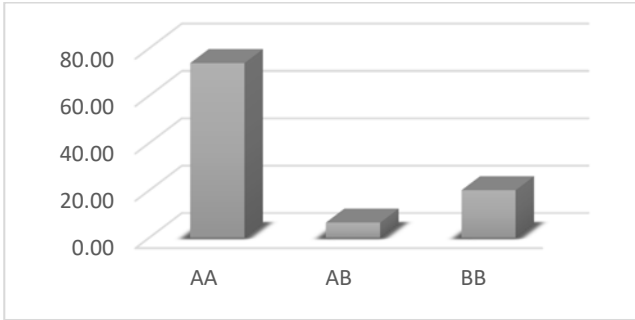

Figure 4 Genotype frequencies for *CAST* gene on TBH breed percent from total population

Table 5 Expected and observed genotypes *CAST* gene at TBH breed

Genotype	Observed	Expected
AA	70	56
AB	6	34
BB	19	5
χ^2 at 1 DF and 5% significance degree	3.85	3.85 < 64,27*
χ^2 calculated	64.27	

*Null hypothesis is denied

CONCLUSIONS

Echo graphic parameters showed in these two measurements points at *Longissimus Dorsi* muscle that TBH lambs have a great potential for meat production, especially illustrated by very good correlations between muscle depth with eye muscle area and also with body weight at birth and 3.5 month age. Eye muscle area on TBH lambs was situated in the limits known for echo graphic parameters specific for meat carcass quality and this is a great opportunity to reveal genetic potential of this breed for carcass meat quality. *CAST* gene polymorphism showed tree variants and *AA* allele was on the first place with 73.68% followed by *BB* with 20% and only with 6.32% for heterozygote variant *AB*. *CAST* genotype showed Hardy Weinberg disequilibrium emphasize a potential empiric selection detrimental for genotype AB. The study will continue with the *CAST* gene effect and the influence on the carcass quality in order to improve the selection criteria and to keep for reproduction the best individuals.

ACKNOWLEDGEMENTS

This study was supported by PN 09-38 project granted by Ministry of Education and Scientific Research.

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